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The effects of led-mediated-photobiomodulation therapy on newly formed bone in distraction osteogenesis

LED-Mediated-photobiomodulation therapy in distraction osteogenesis

Hüseyin Akçay[1], Osman A. Etöz[2], Enis Güray[3], Mustafa Cihat Avunduk[4], Alper Alkan[5]
[1] Department of Oral & Maxillofacial Surgery, Izmir Katip Celebi University, Faculty of Dentistry, İzmir
[2]Private Practice, Kayseri, [3] Private Practice, Ankara
[4]Department of Pathology, Necmettin Erbakan University, Faculty of Meram Medicine, Konya
[5] Department of Oral & Maxillofacial Surgery, Bezmialem University ,Faculty of Dentistry, İstanbul, Turkey

Abstract
Aim: The purpose of this experimental study was to determine the effects of light emitting diode-mediated-photobiomodulation therapy (LPT) on newly formed bone in mandibular distraction osteogenesis (DO). Materials and Methods: Sixteen adult male New Zealand white rabbits were involved in the study. Osteotomy was done on left mandibular corpus under general anesthesia. Custom-made external distractors were positioned to left mandibles of animals. The latency period was 5 days, then distractors were activated twice a day for 7 days with 0,5mmx2/day frequency. Sixteen rabbits were randomly divided into experimental (n=8) and control (n=8) groups. Animals in the experimental group were exposed to LPT with an energy density of 20mW/cm2 for 21 consecutive days directly over the distraction area starting with the distraction period. DO was performed without further treatment in the control group. After 30 days of consolidation period, the animals were sacrificed and samples were harvested. Bone mineral density (BMD) and bone mineral content (BMC) of bone formed through DO were evaluated using dual-energy x-ray absorptiometry (DEXA) and bone samples were processed for histological investigation. The data were analyzed using the Student t-test and the Mann-Whitney U test (p=0.05).Results: Bone mineral density was higher in the distraction gap of the experimental group (p=0.013). The number of osteoblasts and new bone forming area were significantly greater in the experimental group than the control group (p<0.05). Discussion: The results showed that LPT had a positive effect on the biomodulation of newly formed bone in DO in a rabbit model. Photobiomodulating effects of LLLT and LPT on bone healing seem similar according to the literature and LPT may be a safe and useful alternative for accelerating the treatment process of DO.

Keywords
Bone Formation; Distraction Osteogenesis; LLLT; Photobiomodulation Therapy

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Corresponding Author: Hüseyin Akçay, Department of Oral & Maxillofacial Surgery, Izmir Katip Celebi University, Faculty of Dentistry, İzmir, Turkey.
E-mail: hus_akcay@yahoo.com GSM: +90.232.325 4040
Corresponding Author ORCID ID: https://orcid.org/0000-0001-7730-8212

Introduction

Distraction osteogenesis (DO) is an effective therapeutic approach alternative to the use of various bone grafts in the treatment of craniomaxillofacial anomalies and deformities and has become an increasingly popular technique in the past two decades [1]. DO is the traction application to the callus formed between bone fragments created with osteotomies to stimulate bone formation by producing stress on the callus via this traction [2]. Separation and consequent osteogenesis between bone sites are obtained by implanted distraction devices in adjacent bone. After the separation of bone segments and defect repair, activation of the distraction device finishes but the distraction device held in place until the osteoid tissue in the distraction gap has mineralized for consolidation. Due to the time length required for achieving extensive bone regeneration, maturation, and consolidation, distraction osteogenesis process may generate discomfort for patient and may result in some complications [3].

Current aims in the surgical area of DO include enhancing bone regeneration and shortening the necessary time for treatment. Several mechanical, chemical and biological treatment options have been evaluated to increase the quality of newly formed bone and shortening the treatment time of DO [4-8].

Near-infrared (NIR) phototherapy uses monochromatic light in the optical region between 600–1000 nm to irradiate tissues without causing destruction and thermal effects [9]. Phototherapy has been used in medicine and dentistry with the increasing popularity since Maiman developed the ruby laser in 1960. Light-emitting diodes (LEDs) working efficiently of biologically active wavelengths introduced as an alternative photon source after lasers.

Light generated using LED arrays or low-level laser (LLL) between 630–1,000 nm has been shown to enhance retinal function in cultured primary neurons [10]. Phototherapy has the physiologic effect on cellular metabolism with NIR photons absorbed by cytochrome C and increased adenosine triphosphate (ATP) production is achieved in the mitochondria by this respiratory chain which allows improved function and metabolism of poorly perfused or intoxicated cells [11]. Photobiomodulation is suggested to enhance wound healing, tissue regeneration and growth, bone regeneration, nerve regeneration, chondral and fibroblastic proliferation, angiogenesis and collagen synthesis [12-20].

Speeding up the consolidation and bone maturation in DO is a challenging clinical aim. The purpose of this study was to determine the effects of LED photobiomodulation therapy (LPT) on bone healing during the consolidation period of DO and the possibility of a shortened consolidation period for earlier device removal and reduction of complications.

Material and Methods

Sixteen adult male 8-month-old New Zealand rabbits (Oryctolagus cuniculus), each weighing between 3000 and 3500 g divided into two groups (n=8) were used for this study.

The rabbits were kept single in standard cages at a temperature of 23°C, exposed to a 12- hour light-dark sequence. They were acclimated for 2 weeks before surgery. All animals were fed with standard laboratory pellet chow and distilled water ad libitum. The study was approved by Erciyes University Animal Care and Ethics Committee. All procedures and follow-up were held according to local animal studies research center regulations.

Surgical procedure

All surgical processes were done under sterile circumstances in an operating room by the same experienced surgeon. Intramuscular (IM) injection of 35 mg/kg ketamine (Ketalar; Pfizer, New York, USA) and 8 mg/kg xylazine hydrochloride (Rompun; Bayer, Leverkusen, Germany) was used to achieve general anesthesia. The animals were then placed on the operating table in a supine position. The operation area was shaved and prepared with betadine solution. Lidocaine (0.5%) with 1:200.000 epinephrine was injected in the left ramus and submandibular areas subcutaneously. A 3 cm incision was completed through the skin on the left of the mandible 2 cm from the midline. The facial artery was conserved to the extent as much as possible during dissection through the subcutaneous and muscle layers to expose the lateral side of the mandibular corpus. The mental nerve was identified and preserved. A vertical corticotomy between the premolar teeth and inferior border of mandible was made with a reciprocating saw and the titanium pins used for fixating the distractor were placed perpendicular to the corpus and parallel to each other (Figure1). A custom-made distractor was fixed to the pins, then the osteotomy was completed with a thin chisel osteotome and the distractor was tested (Figure 2). Soft tissues were primarily closed in layers.

Postoperatively, both groups of animals were given IM 200.000 U of penicillin and 0.2 mg buprenorphine every 12 hours for 3 days. The wound was cleaned with betadine daily during the first week. After a 5-day latency period, the distractor was activated at a rate of 0.5 mm/12 hours for seven days.

The animals were randomly divided into 2 groups. LPT was applied to the distraction area of 8 rabbits in the experimental group for 21 days starting with the operation. The remaining subjects served as controls.

LED photobiomodulation therapy

OsseoPulse® LED device (Biolux Research Ltd., Vancouver, Canada) was used for LPT application with 618 nm wavelength and 20 mW/cm2 output power in the present study for 20 minutes once a day during21 days after stabilizing animals in a plastic holder to ensure the immobility. The treatment array was positioned in contact with the distraction site (Figure 3). All LPT procedures were applied transmucosally by the same operator (H.A.).

Radiologic and Histomorphometric Analysis

After 30 days of consolidation period, all animals were sacrificed by ketamine and xylazine overdose. Mandibles were dissected out and fixed in 10% formalin (Figure 4).

Dual-energy x-ray absorptiometry (DEXA) analysis was used for radiographic assessment. Bone mineral density (BMD) and bone mineral content (BMC) of bone regenerates in the distraction gap, bone around the pins of distractor and sham group which is normal bone in distracted hemimandibles were measured using DEXA (Lunar DPX-IQ, Madison, WI) (Figure 5).

Each hemimandible was kept in a 10% formalin solution for a week. Then the bone specimens were placed in 10% hydrochloric acid to decalcify for a week at 25˚C. After decalcification,

Table 1. Descriptive statistical data and statistical comparison of DEXA analysis in experimental and control groups

	LED Photobiomodulation				Control				P
	Mean	SD	Min	Max	Mean	SD	Min	Max	
BMC_R1	0.1107	0.0132	0.0959	0.1308	0.1061	0.0183	0.0784	0.1407	0.586
BMC_R2	0.0462	0.0054	0.0409	0.0550	0.0426	0.0068	0.0286	0.0515	0.281
BMC_R3	0.0208	0.0029	0.0163	0.0252	0.0246	0.0038	0.0196	0.0317	0.051
BMD_R1	0.4195	0.0499	0.3632	0.4956	0.3974	0.0577	0.3142	0.5152	0.446
BMD_R2	0.3897	0.0329	0.3557	0.4490	0.3144	0.0619	0.1818	0.3961	0.013
BMD_R3	0.3557	0.0497	0.2793	0.4316	0.3341	0.0851	0.1572	0.4523	0.568

SD: Standard Deviation; BMC: Bone Mineral Content (gr); BMD: Bone Mineral Density (gr/cm2) R1: sham group (healty bone), R2: Distraction Gap, R3: Bone around pins

Table 2. Descriptive statistical data and statistical comparison of histomorphometric analysis in experimental and control groups (osteoblast-new bone formation)

	LED Photobiomodulation				Control				P
	Mean	SD	Min	Max	Mean	SD	Min	Max	
Osteoblast	50.14	7.537	41	61	21.75	5.12	12	29	0.000
New Bone	204042	41970	169410	269700	123264	24902	82234	157701	0.000

Table 3. Descriptive statistical data and statistical comparison of histomorphometric examination of experimental and control groups (osteoclast-vessel)

	LED Photobiomodulation			Control			P
	25%	Median	75%	25%	Median	75%	
Osteoclast	3.00	4.00	4.00	3.00	4.00	4.75	0.951
Vessel	2.00	5.00	5.00	2.00	3.00	4.50	0.303

the distraction field was segmented with a scalpel to form a perpendicular slice of mandible. The bone sections were rinsed, trimmed, and implanted in paraffin. The paraffin blocks were sectioned consecutively at 5-µm thickness and stained with hematoxylin and eosin.

All specimens were examined with a light microscope (Nikon Eclipse E400) and a photograph attachment (Nikon Coolpix 5000) was used to photograph each specimen. All images were then transported to PC environment and evaluated with an image analysis program (Clemex Vision Lite Image Analysis 3.5; Clemex Technologies, Longueuil, Canada). Using this analysis program, 0.5 mm2 areas were designated. Excluding damaged cells, vessels, osteoblasts and osteoclasts were marked in the area. With the same image analysis program, the marked cells are automatically calculated. Furthermore, new bone- forming areas were determined and assessed (µm2) in 0.5 mm2 area.

Statistical analysis

All data were analyzed using SPSS (Statistical Package for Social Science, Version 20.0, SPSS Inc., Chicago, IL, USA) package program. The normality and the homogeneity of the data were evaluated using the Kolmogorov-Smirnov normality test and the Levene's variance homogeneity test. For the osteoclast number and new forming vessel number, the data were not normally distributed thus, the Mann-Whitney U test was used to compare groups. For other variables, the Student t-test was used. The level of statistical significance was set at p less than 0.05.

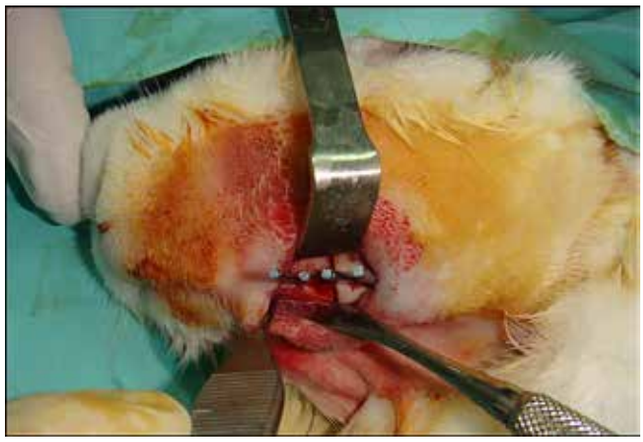

Figure 1. A vertical corticotomy line between the premolars and pins

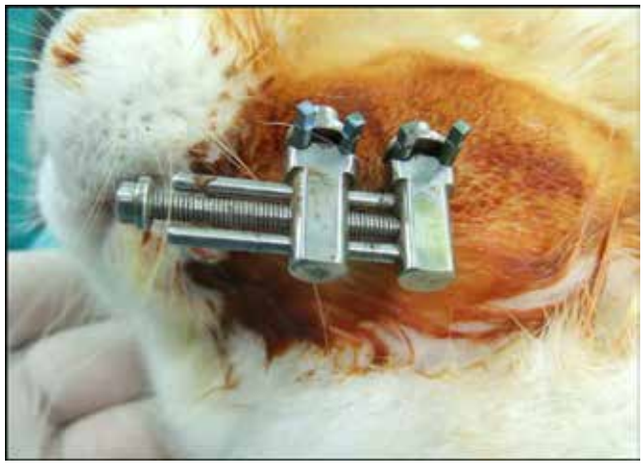

Figure 2. A custom made distraction device fixed and tested

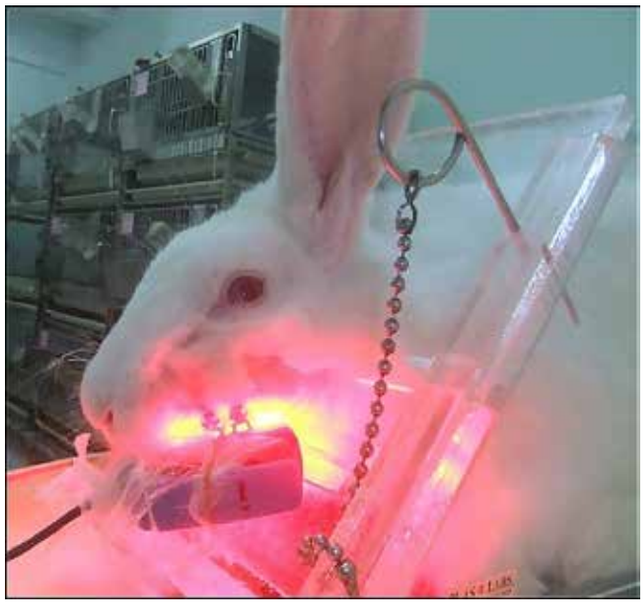

Figure 3. LED application

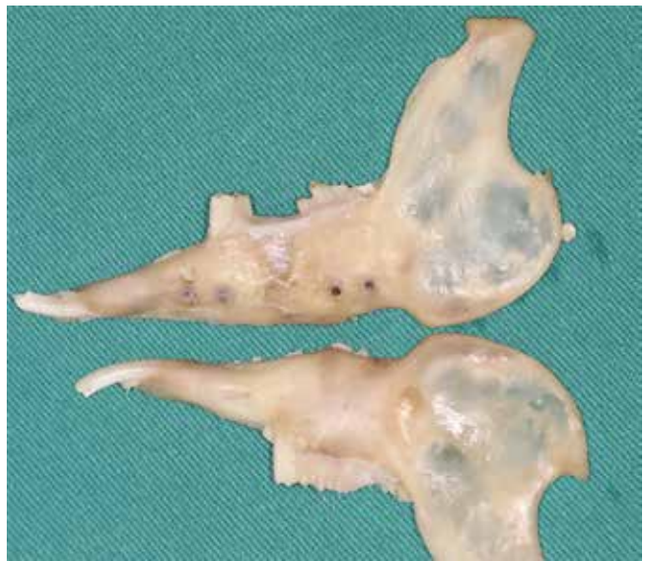

Figure 4. Mandibles of the animals were removed and cut into 2 parts from the midline

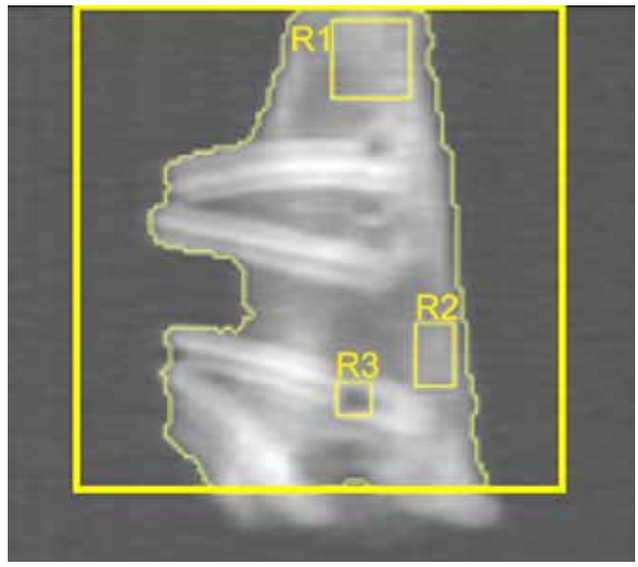

Figure 5. DEXA was used to measure the bone mineral density (BMD) and bone mineral content (BMC) of bony regenerates, R1 indicate the undamaged bone area, R2 indicate new bone forming area, R3 indicate the bone around the pin and distraction device.

Results

One animal from the control group was infected and excluded from the study during the consolidation period. A total of 15 hemimandibles which were subjected to DO method were evaluated ($p>0.05$, Table 1, 2, 3).

When DEXA data were analyzed, it was seen that bone mineral density values were significantly higher in the distraction area of the experimental group ($p=0.013$, Table 1). However, no significant difference was observed in DEXA data in adjacent bone areas or pin circumferences ($p>0.05$, Table 1).

Histomorphometric evaluation showed significant increase of osteoblasts and new bone forming area in experimental group ($p<0.05$, Table 2, Figure 6). In terms of the number of vessels, although the number in the experimental group was higher, it was not statistically significant ($p>0.05$, Table 3).

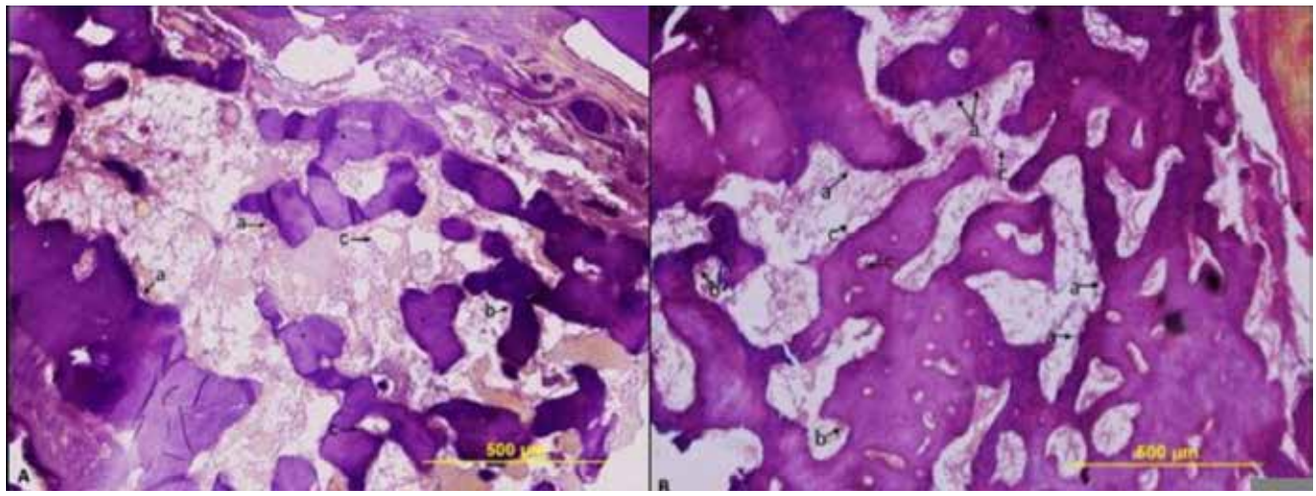

Figure 6. Histologic specimens of control group (A) and LED photobiomodulation group (B) showing a-osteoblast, b-osteoclast, c-vessels, (H&E x 100)

Discussion

The use of DO in the treatment of various bone losses and congenital or acquired deformations has become common in maxillofacial surgery in last decades. The main factors affecting the success of the DO are a series of technical factors, such as latency period, distraction rate, rhythm and consolidation length. Long treatment time may lead to various complications during DO [3]. The study reported by Ilizarov stated that a latency phase of at least 7 days is required in adults and could be shorter in pediatric patients. Various studies have suggested different protocols on distraction rates and latency phases [1]. In a study conducted by Aida et al. [21] on the rabbit DO model, it was reported that mature callus with matured lamellar bone is detectable after 4 weeks thus a consolidation period of 1–2 months is appropriate. Currently, a latency period of up to 7 days and a distraction rate of 1 mm per day are considered to be standard in the treatment of adults by DO in clinical practice. Reducing the time of latency and consolidation periods has advantages such as reduced risk of infection and treatment failure. The number of DO researches about shortening the treatment time is increasing. The effects of various pharmacological agents, biostimulation methods, and mechanical stimulation applications to enhance new bone formation during DO were assessed by different researchers.

In the current study, we analyzed the effect of LPT on the quality of osteogenesis during mandibular DO in the rabbits. Although there are studies that investigated the effects of LPT in various surgical procedures, no studies were performed investigating LPT in DO to our knowledge.

Ekizer et al. [14] investigated the efficiency of LED photobiomodulation on bone formation in interpremaxillary suture during orthopedic expansion and showed that it had a stimulating effect on bone formation. El-Bialy et al. suggested that photobiomodulation therapy stimulated mandibular growth in rats [22]. The efficiency of LPT on the rate of orthodontic movement was evaluated by clinical and experimental trials [14,23]. Kau et al. [23] reported that LPT application at 850-nm near-infrared wavelength, increased the rates of orthodontic movement in the alignment phase of treatment clinically. In a human model, more rapid achievement of dental implant stability was demonstrated as a result of the biostimulation effect of LPT [24].

Although there is no study investigating effects of LPT on DO in the literature, there are limited studies about the effect of LLLT. Miloro et al. evaluated the effect of LLLT achieved by 820 nm wavelength GaAlAs laser in the rabbit model and reported that bone formation was higher in LLLT groups than the control groups [15]. Abd-Elaal et al. evaluated the influence of GaAs laser on mandibular DO clinically and reported that bone quality and quantity was higher in the LLLT group than the control group. They offered that LLLT may shorten treatment process [20]. Results of our study in which biostimulation was achieved with an LED source, appear to be similar to LLLT in new bone formation during DO.

The wavelength of irradiation formed by LED is similar to that expended in LLL (600–1,000 nm) and photobiomodulating effects were supplied with both sources. Laser and LED irradiation stimulate the electron transfer in Cytochrome C Oxidase and increase the intracellular ATP production [10]. LPT has been shown to hasten tissue healing and upturn from ischemia due to increased collagen production and angiogenesis by increasing mitochondrial activity and ATP synthesis [25]. Based on these cellular outcomes, LPT may be effective for the increased bone formation in DO. In the current study, LPT was applicated by a LED device with 618nm wavelength and quality of the new forming bone was evaluated during DO.

LED light is not coherent as laser light, then expected to cause less side effects [9]. LED radiation can be created at a lower price in comparison to the LLL, and applicable to a large area of the body surface safely. Other advantages of LEDs over lasers for use in phototherapy include littler hardware package, lower energy density, and reduced eye damage risk. [11]. In addition, the use of LED arrays provides the opportunity to expand the footprint allowing a one-time application for wide surfaces.

In the present study, LPT improved the bone formation significantly according to radiographic and histologic evaluations. BMD values on the 30th day of the consolidation phase were found to vary significantly between the experimental group

subjected for LPT in a wavelength of 618 nm and control groups which are in viable with those obtained in similar studies [17,18]. BMD values in the LED group were also consistent with those obtained histopathologically. Photobiomodulation application may provide a clinical advantage by accelerating bone healing during DO as the complications rise according to the treatment time. On account of accelerating bone healing and decreasing treatment time by using LPT, satisfaction and cooperation of the patients can be enhanced.

Conclusions

There are some studies related to effects of photobiomodulation on bone healing in the literature, but there are none with the use of the infrared LED as the light source in DO. In this study, LED photobiomodulation significantly has positive effect on bone healing on newly formed bone in DO according to radiologic and histologic evaluations in a rabbit model. The role of biological and biomechanical variables influencing angiogenesis and mineralization should be determined to improve the best method to hasten bone healing. Further clinical and experimental studies using LED photobiomodulation with bone substitutes should be investigated.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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